



TITLE:

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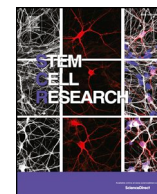
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Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line, BRCi001-A, derived from a patient with mucopolysaccharidosis type I



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ABSTRACT

Mucopolysaccharidosis type I (MPS I) is a rare inherited metabolic disorder caused by defects in alpha-L-iduronidase (IDUA), a lysosomal protein encoded by *IDUA* gene. MPS I is a progressive multisystemic disorder with a wide range of symptoms, including skeletal abnormalities and cognitive impairment, and is characterized by a wide spectrum of severity levels caused by varied mutations in *IDUA*. A human iPSC line was established from an attenuated MPS I (Scheie syndrome) patient carrying an *IDUA* gene mutation (c.266G > A; p.R89Q). This disease-specific iPSC line will be useful for the research of MPS I.

Resource table

Unique stem cell line identifier	BRCi001-A
Alternative name of stem cell line	HPS0660
Institution	RIKEN BioResource Research Center (BRC), Kyoto, Japan
Contact information of distributor	Haruhisa Inoue haruhisa.inoue@riken.jp
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 47 years of age Sex: female Ethnicity: Japanese
Cell source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Episomal vectors (SOX2, KLF4, OCT4, L-MYC, LIN28, p53 carboxy-terminal dominant-negative fragment (mp53DD), EBNA1)
Genetic modification	Yes
Type of modification	Hereditary
Associated disease	Mucopolysaccharidosis type I (MPS I, Scheie syndrome)
Gene/locus	<i>IDUA</i> gene/Chromosome 4
Method of modification	Not available
Name of transgene or resistance	Not available
Inducible/constitutive system	Not available
Date archived/stock date	Dec 2014
Cell line repository/bank	RIKEN BioResource Research Center (BRC), Japan http://en.brc.riken.jp/index.html cellbank.brc@riken.jp
Ethical approval	Ethics Committee of the RIKEN BioResource Research Center (approved No. Tsukuba 29-1) Ethics Committee of the Department of Medicine and the Graduate School of Medicine of Kyoto University (approved No. R0091, G259)

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Resource utility

MPS I is an autosomal recessive disorder caused by mutations of the *IDUA* gene. The disease pathophysiology is not well understood. iPSCs were generated from an MPS I patient with an *IDUA* gene mutation (c.266G > A; p.R89Q). These disease-specific iPSCs will be used to study the pathological mechanisms of MPS I.

Resource details

Mucopolysaccharidosis Type I (MPS I) is a lysosomal storage disorder caused by mutations of the alpha-L-iduronidase (*IDUA*) gene (Poletto et al., 2018; Yamagishi et al., 1996). The condition affects many parts of the body, including the brain, musculoskeletal system, heart, lungs, and eyes. Mutations of the *IDUA* gene lead to *IDUA* enzyme deficiency, resulting in the accumulation of glycosaminoglycans (GAGs), heparan sulfate and dermatan sulfate in cells and especially in lysosomes. This causes many organs and tissues of the body to become

enlarged in MPS I. More than one hundred different mutations of the *IDUA* gene have been identified in individuals with MPS I. These variations result in a broad range of severity levels and disease progression patterns among individuals with MPS I (Poletto et al., 2018; Yamagishi et al., 1996). However, the disease pathophysiology is largely unknown. MPS I-specific iPSCs can be used to understand the pathophysiological mechanism of MPS I. We established and characterized an iPSC line from a patient with attenuated MPS I who carried an *IDUA* gene mutation (c.266G > A; p.R89Q). MPS I-specific iPSCs were generated from peripheral blood mononuclear cells (PBMCs) of an MPS I patient using episomal vectors carrying reprogramming factors under feeder-free culture conditions (Nakagawa et al., 2014; Okita et al., 2013). The MPS I-specific iPSC (HPS0660) presented a human ES cell-like morphology. The iPSCs were found to be positive for pluripotent marker NANOG and SSEA4 by immunocytochemistry (Fig. 1A, green: NANOG, red: SSEA4), and 99.9% of the iPSCs were found to be positive for pluripotent marker SSEA4 by flow cytometry. The three-germ-layer differentiation capacities of the iPSCs were revealed with an *in vitro* EB

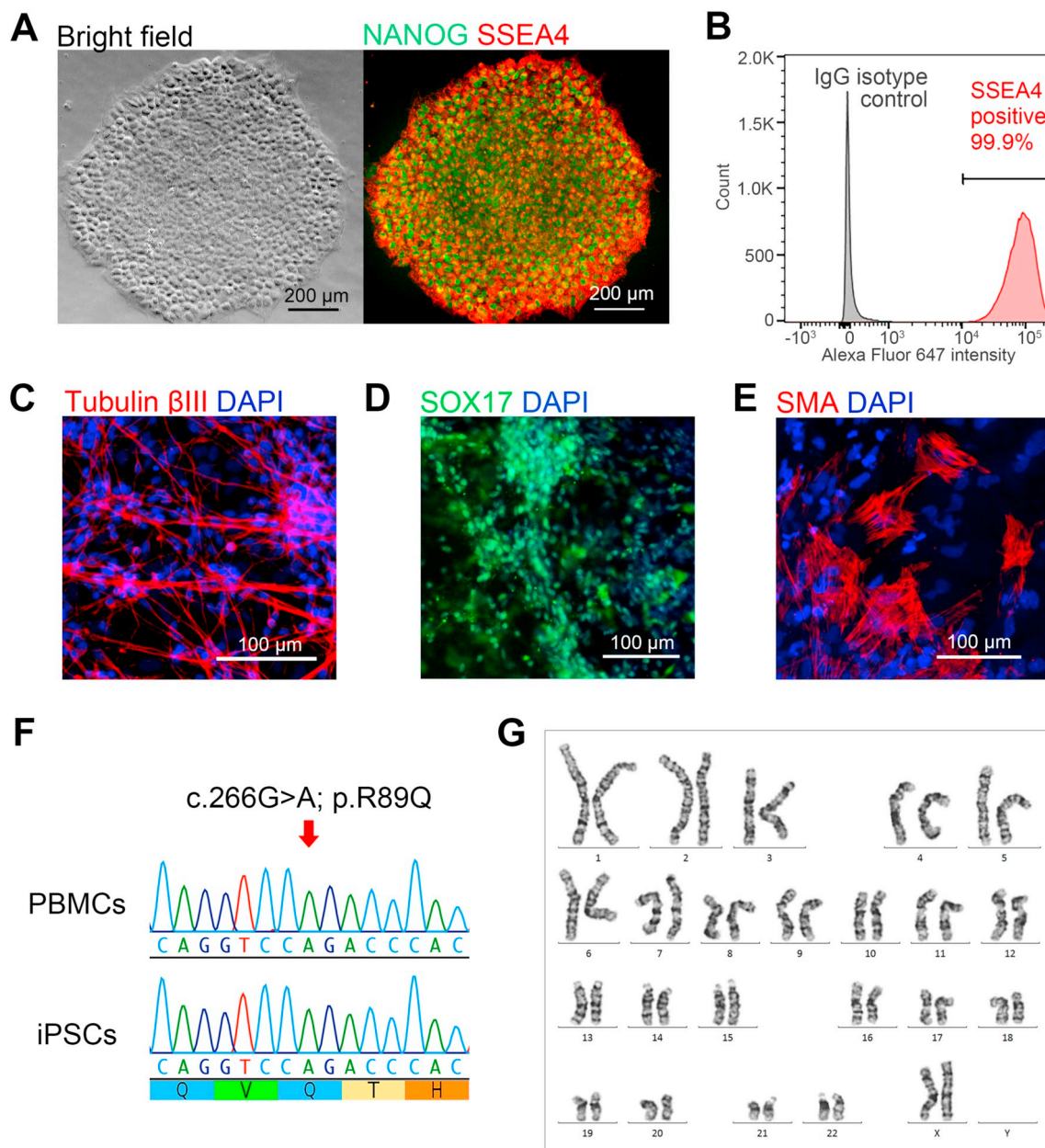


Fig. 1. Characterization of the iPSC line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology Phenotype	Photography	Normal, human ESC like morphology	Fig. 1 panel A
	Qualitative analysis (Immunocytochemistry)	Positive staining of pluripotency markers, NANOG, SSEA-4	Fig. 1 panel A
	Quantitative analysis (Flow cytometry)	99.9% of positive cells for pluripotency cell surface marker SSEA-4	Fig. 1 panel B
Genotype Identity	Karyotype (G-banding) and resolution	46XX, Resolution 400	Fig. 1 panel G
	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	16 loci, matched	Available with the authors
Mutation analysis (if applicable)	Sequencing	Homozygous <i>IDUA</i> mutation (c.266 G > A, p. R89Q)	Fig. 1 panel F
	Southern Blot or WGS	Not performed	Not performed
	Microbiology and virology	Mycoplasma testing by indirect DNA fluorescent staining and RT-PCR. Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation	Proof of three-germ-layer formation: Tubulin β III (ectoderm), SOX17 (endoderm) and smooth muscle actin (mesoderm)	Fig. 1 panel C, D and E
Donor screening (OPTIONAL) Genotype additional info (OPTIONAL)	HIV, HTLV-1, Hepatitis B, Hepatitis C	Negative	Available with the authors
	Blood group genotyping	Not available	Not available
	HLA tissue typing	Not available	Not available

formation assay (Fig. 1C, Tubulin β III: ectoderm, D, SOX17: endoderm, and E, smooth muscle actin (SMA): mesoderm). Furthermore, the iPSCs retained the *IDUA* gene mutation and normal karyotype. The identity of the cell line was verified with an STR analysis. Mycoplasma contamination was not detected in the cell culture.

Materials and methods

Ethics statement

The generation and use of human iPSCs was approved of by Ethics Committees of RIKEN BioResource Research Center, Department of Medicine and Graduate School of Medicine of Kyoto University. All methods used in this study were performed in accordance with approved guidelines. Formal informed consent was obtained from the patient.

Establishment of iPSCs

The iPSCs were generated from peripheral blood mononuclear cells (PBMCs) obtained from a patient with attenuated MPS I, according to the protocol for the generation and culture of human iPSCs under feeder-free conditions (Nakagawa et al., 2014, <http://www.cira.kyoto-u.ac.jp/e/research/protocol.html>). In brief, the PBMCs were cultured for 5 days in MNC medium; StemSpan-ACF medium (STEMCELL Technologies, Vancouver, Canada), supplemented with 100 ng/ml rhSCF, 100 ng/ml rhTPO, 100 ng/ml rhFlt3L, 50 ng/ml rhIL-6, 20 ng/ml rhIL-3 (all from R&D Systems, Minneapolis, MN). The episomal vectors (pCE-hOCT3/4 [0.63 μ g], pCE-hSK [SOX2 and KLF4, 0.63 μ g], pCE-hUL [L-MYC and LIN28, 0.63 μ g], pCE-mp53DD [p53 carboxy-terminal dominant-negative fragment, 0.63 μ g], and pCXB-EBNA1 [0.5 μ g]) were transduced into PBMCs (3,000,000 cells) by electroporation using the Nucleofector 2b Device with a Human CD34+ Cell Nucleofector Kit (Lonza, Basel, Switzerland). The electroporated cells were plated in MNC medium on iMatrix-511 E8 fragment (MATRIX-OME, Osaka, Japan)-coated cell culture plates and cultured in a CO₂ incubator at 37 °C under 5% CO₂. For two to eight days after the electroporation, an equal volume of StemFit medium was added to the culture wells every other day without aspiration, then the culture medium was replaced with StemFit medium to induce iPSCs. The cells were cultured in the CO₂ incubator at 37 °C under 5% CO₂ for 14–18 days until iPSC colonies were observed. The selected iPSC clone was expanded and maintained using StemFit medium and iMatrix-511 E8 fragment (Nakagawa et al., 2014). The iPSCs were single-cell passaged every 6–8 days using 0.5 \times TrypLE Select (TrypLE Select (Gibco, Thermo Fisher, Waltham, MA) diluted 1:1 with 0.5 mM EDTA solution). The plating density was 1350 cells per cm². 10 μ M Y-27632 (Wako,

Osaka, Japan) was added to StemFit medium when the iPSCs were passaged, and was removed by replacing the medium with fresh StemFit medium the next day.

Karyotyping

A G-band analysis was performed by LSI Medience (Tokyo, Japan) to determine the karyotype of the iPSC line at passage number 6. A total of 20 metaphases were analyzed.

Genotyping

Genomic DNA of undifferentiated state iPSCs at passage number 4 and the parental PBMCs was extracted using a PureLink Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific) and amplified by a PCR using the enzyme KOD Plus Neo (TOYOBO, Japan) and Veriti thermal cycler (Applied Biosystems, Thermo Fisher Scientific). The thermocycling conditions were as follows: initial denaturation at 94 °C for 2 min, 35 cycles of 2-step thermocycling (denaturation at 98 °C for 10 s and extension at 68 °C for 30 s), and hold at 10 °C. The targeted PCR product was extracted from agarose gel using a PureLink Quick Gel Extraction Kit (Invitrogen, Thermo Fisher Scientific) and then subjected to direct sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific) according to manufacturers' instructions. A 3500xL Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific) was used for the sequencing analyses. The primer set used for genotyping is listed in Table 2.

In vitro three-germ-layer differentiation assay

An embryoid body (EB) formation assay was performed to differentiate iPSCs into three germ layers. EBs were formed from the 0.5 \times TrypLE Select-dissociated iPSCs (9000 cells per EB) in DMEM/F12 medium supplemented with 20% KSR, 2 mM L-glutamine, 0.1 mM NEAA, 0.1 mM 2-mercaptoethanol (All from Gibco, Thermo Fisher Scientific), 10 μ M Y-27632 (Nacalai Tesque, Kyoto, Japan) using a low-attachment surface V-bottom 96-well plate (Sumitomo Bakelite, Tokyo, Japan) for 11 days, and then cultured in DMEM containing 10% FBS (Gibco, Thermo Fisher Scientific) on a Matrigel (BD Bioscience)-coated tissue culture plate for 7 days Table 1.

Immunocytochemistry

Indirect fluorescent immunostaining was performed. Cells were fixed in 4% paraformaldehyde (Nacalai Tesque) in PBS for 20 min at room temperature. For permeabilization and blocking, the fixed cells

Table 2
Reagent details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Rabbit anti-NANOG	1:500	Cosmo Bio Co Cat# RCAB0003P, RRID: AB_1962353
Pluripotency marker	Mouse anti-stage-specific embryonic antigen-4 (SSEA4)	1:1000	Millipore Cat# MAB4304, RRID: AB_177629
Differentiation marker (Ectoderm)	Mouse anti-Tubulin β III	1:1000	Millipore Cat# MAB1637 RRID: AB_2210524
Differentiation marker (Endoderm)	Goat anti-SOX17	1:1000	R&D systems Cat# AF1924 RRID: AB_355060
Differentiation marker (Mesoderm)	Mouse anti-alpha-Smooth Muscle Actin (SMA)	1:500	DAKO Cat# M0851, RRID: AB_2223500
Secondary antibody	Goat anti-Rabbit IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11034, RRID: AB_2576217
Secondary antibody	Goat anti-Mouse IgG Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-11030, RRID: AB_2534089
Secondary antibody	Donkey anti-Goat IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11055 RRID: AB_2534102
Pluripotency marker	Alexa Fluor 647 Mouse anti-SSEA4	1 μ g/test	BD Biosciences Cat# 560796 RRID: AB_2033991
Isotype control	Alexa Fluor 647 Mouse IgG3, k Isotype Control	1 μ g/test	BD Biosciences Cat# 560803 RRID: AB_2034029

Primers		
	Target	Forward/Reverse primer (5'-3')
Genotyping	<i>IDUA</i> gene sequencing (498 bp)	CTTGAACGTGTGTGTCAGCC/GACACACACAGGGATGCTCA
Nested-PCR, 1st step PCR (MCGpF11/MCGpR1)	mycoplasma detection (200–400 bp)	ACACCATGGGAG(C/T)TGGTAAT/CTTC(A/T)TCGACTT(C/T)CAGACCAAGGCAT
Nested-PCR, 2nd step PCR (R16–2/MCGpR21)	mycoplasma detection (200–400 bp)	GTG(C/G)GG(A/C)TGGATCACCTCCT/GCATCCACCA(A/T)A(A/T)AC(C/T)CTT

were incubated with blocking buffer: PBS containing 5% Blocking One (Nacalai Tesque) and 0.2% Triton-X 100 (Nacalai Tesque). The primary and secondary antibodies used in this study are listed in Table 2. Primary antibodies and secondary antibodies were reacted in the blocking buffer for 16 h (overnight) at 4 °C and for 1 h at room temperature, respectively. Nuclei were stained with DAPI (Invitrogen, Thermo Fisher Scientific). Images were obtained with a BZ-X710 microscope (Keyence, Osaka, Japan).

Flow cytometry

The iPSCs were dissociated with Accumax solution (Sigma Aldrich, St. Louis, MO). The dissociated iPSCs were suspended in PBS containing 2% FBS (Stain buffer, BD Bioscience, San Jose, CA) at a density of 1.0×10^6 cells/ml. The cells were stained for 30 min on ice and analyzed with FACS Aria (BD Bioscience). The Alexa Fluor 647-labeled antibodies used for flow cytometry are listed in Table 2. The FlowJo software program (ver.10, FLOWJO, BD Bioscience) was used for the data analysis.

STR analysis

Genomic DNA was extracted from the iPSCs and PBMCs using a PureLink Genomic DNA Mini Kit and subjected to an STR analysis using PowerPlex® 16 System (Promega, Madison, WI).

Mycoplasma test

iPSCs were confirmed to be mycoplasma-negative by indirect DNA fluorescent staining and a nested-PCR. iPSC culture medium was tested by staining with Hoechst33258 (Thermo Fisher Scientific) after 5–6 days of co-culture with VERO cells (RCB0001, RIKEN BRC Cell Bank) as mycoplasma negative indicator cells. DNA was extracted and subjected to a nested-PCR. The primers used in the PCR are listed in Table 2. AmpliTaq Gold 360 DNA Polymerase (Thermo Fisher Scientific) was used for each step of the PCR. The same thermocycling conditions were used for both steps: initial denaturation of 10 min at 95 °C, 30 cycles of thermocycling; 30 s at 95 °C; 2 min at 55 °C; 2 min at 72 °C, final extension of 5 min at 72 °C, and hold at 10 °C. The PCR products were detected by electrophoresis on 2% agarose gel and ethidium bromide staining.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2019.101406>.

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References

- Nakagawa, M., Taniguchi, Y., Senda, S., Takizawa, N., Ichisaka, T., Asano, K., Morizane, A., Doi, D., Takahashi, J., Nishizawa, M., Yoshida, Y., Toyoda, T., Osafune, K., Sekiguchi, K., Yamanaka, S., 2014. A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells. *Sci. Rep.* 4, 3594. <https://doi.org/10.1038/srep03594>.
- Okita, K., Yamakawa, T., Matsumura, Y., Sato, Y., Amano, N., Watanabe, A., Goshima, N., Yamanaka, S., 2013. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. *Stem Cells* 31, 458–466. <https://doi.org/10.1002/stem.1293>.
- Poletto, E., Pasqualim, G., Giugliani, R., Matte, U., Baldo, G., 2018. Worldwide distribution of common IDUA pathogenic variants. *Clin. Genet.* <https://doi.org/10.1111/cge.13224>.
- Yamagishi, A., Tomatsu, S., Fukuda, S., Uchiyama, A., Shimozaawa, N., Suzuki, Y., Kondo, N., Sukegawa, K., Orii, T., 1996. Mucopolysaccharidosis type I: identification of common mutations that cause Hurler and Scheie syndromes in Japanese populations. *Hum. Mutat.* 7, 23–29. [https://doi.org/10.1002/\(SICI\)1098-1004\(1996\)7:1<23::AID-HUMU3>3.0.CO;2-Q](https://doi.org/10.1002/(SICI)1098-1004(1996)7:1<23::AID-HUMU3>3.0.CO;2-Q).